Quality Assurance Plan

Maine Department of Marine Resources
Bureau of Resource Management
Division of Public Health
Biotoxin Monitoring Laboratories

Boothbay Harbor and Lamoine, Maine

Revised, April 2007
Darcie A Couture

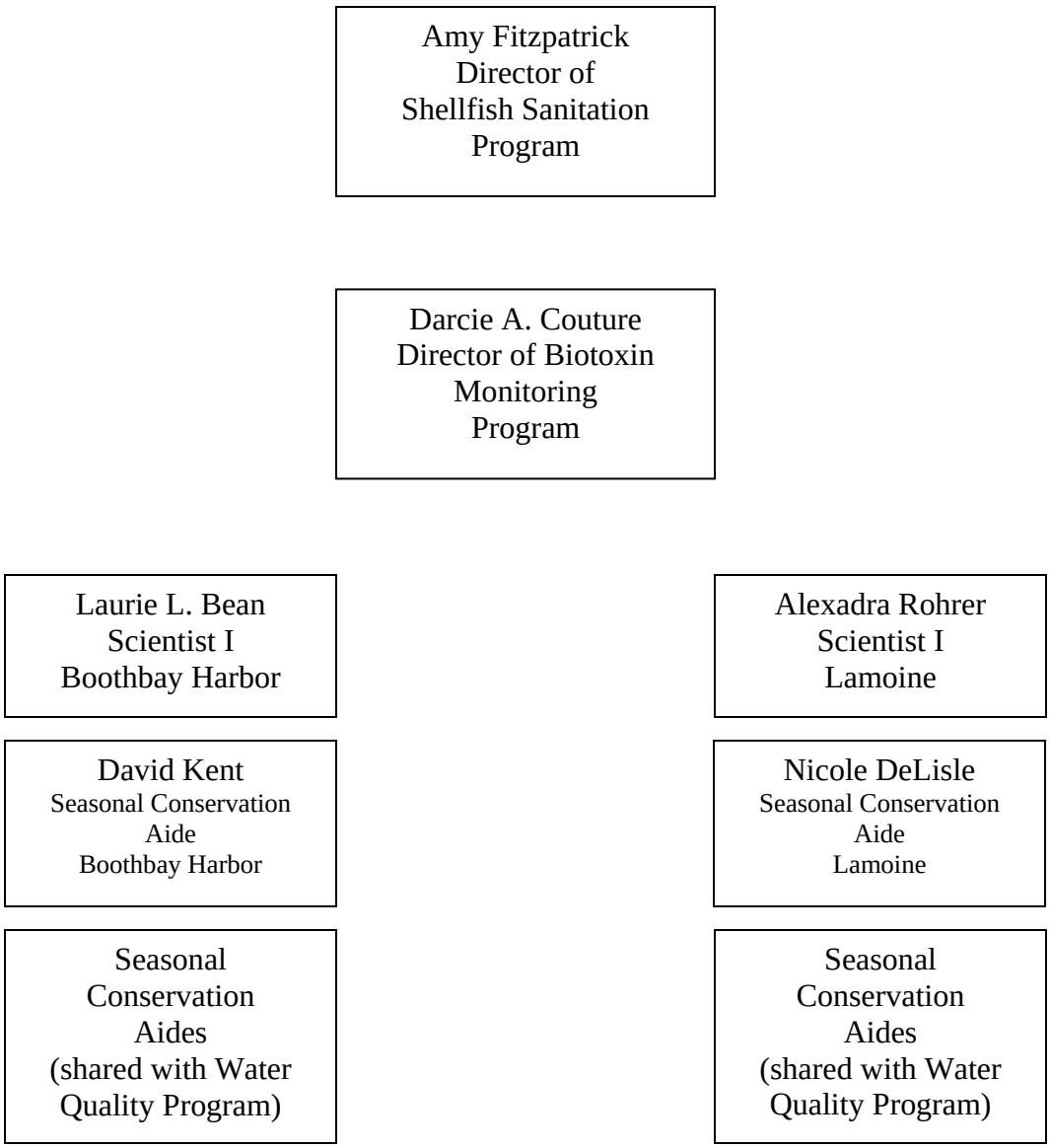
Contents

I.

Organization of the Laboratory

ORGANIZATION OF THE LABORATORY

Department of Marine Resources
Biotoxin Monitoring 2006



The State of Maine Department of Marine Resources Biotoxin Monitoring Program is serviced by two laboratories. There is a laboratory in each of the district offices, in the western part of the state at West Boothbay Harbor and in the eastern part of the state at Lamoine State Park. All shellfish samples tested for Paralytic Shellfish Poisoning (PSP) for the National Shellfish Sanitation Program (NSSP) are run in these laboratories.

The Director of Shellfish Sanitation supervises the activities of both laboratories. Each lab is staffed by a Marine Resources Scientist I, a permanent Seasonal Conservation Aide, and one or more Seasonal Conservation Aides which are shared with the Water Quality Program.

Sample collection for PSP is done by the Scientists and Conservation Aides. Occasionally Water Quality staff, Marine Patrol officers, Area Biologists, Seafood Inspectors, and other specially licensed personnel assist in the collection of samples. Sampling for PSP is conducted from mid March into late October for most species, although surf clam sampling may occur year-round, as needed.

The Laboratory duties in both the Boothbay Harbor and Lamoine laboratories are shared by the Scientists and the Conservation Aides. The shared work includes collecting and preparing samples, calibrating equipment, performing required quality control, and caring for the mice. The Scientists are responsible for PSP data entry, responding to requests for data from other programs, states, and countries, and coordinating sampling. Scientists must also train staff, inventory and order supplies, order mice, maintain equipment, and ensure that the laboratory is operating within the guidelines of the DMR Quality Assurance Plan and the NSSP Laboratory Certification Program. The Director of Biotoxin Monitoring oversees and guides sample scheduling, reviews all toxicity data, and is responsible for implementing the Administrative Procedures Act for promulgation of shellfish harvesting area closures and openings. The Director of Biotoxin Monitoring also maintains close contact with other states and countries regarding current toxicity levels, represents the Department at meetings, and is the contact point for media, industry, and any other public inquiries regarding the Biotoxin Monitoring Program.

The Department's Biotoxin Monitoring Program is mandated by 12 M.R.S.A. Sections 6172 and 6192. Compliance with the NSSP is overseen by the U.S. Food and Drug Administration.

Contact information:
Linda Chandler
US Food and Drug Administration
Office of Compliance
HFS – 626
5100 Paint Brush Parkway
College Park
MD 20740-3835
Phone 301-436-2151
FAX 301-436-2672

II.

Staff Training Requirements

All persons working in the laboratory must be proficient in laboratory skills, safety practices, standard operating procedures, quality control measures, and equipment calibration and maintenance. The laboratory quality assurance plan delineating each of these areas is available for everyone's use and every laboratory employee should be familiar with it. It is the responsibility of the Scientists to train all staff members in the above stated skills. Employees are encouraged to take training that is pertinent and available and to attend workshops and seminars.

All persons working in the field must be familiar with the sampling stations, safety, standard operating procedures, and quality control procedures of field collection. It is the responsibility of the Scientists to train all staff members in the above stated skills.

All staff members are expected to behave responsibly and professionally in the workplace. If a problem or question arises, the staff is required to seek assistance from their direct supervisor immediately. Failure to behave responsibly is grounds for progressive disciplinary action or termination.

Each new employee must be proficient in all of the skills listed on the new employee checklist (Appendix I.), in order to work independently of supervision.

Laboratory staff are evaluated yearly using the State of Maine's Performance Appraisal system. Copies are discussed with and given to employees and maintained on file in the Department's Hallowell office.

III.

Standard Operating Procedures

Introduction

The integrity of laboratory results is greatly affected by the handling of the samples from time of collection to time of analysis, as well as the preparation and handling of all chemicals and equipment involved in the extraction process and the acclimation and handling of the mice used in the bioassay. It is imperative that the sample does not deteriorate or become contaminated before it reaches the laboratory. The FDA itemizes, in the Shellfish Laboratory Evaluation Checklist, the requirements for the handling and transportation of shellfish samples to maintain the integrity of the samples. This chapter outlines all of the procedures associated with every aspect of the program, from sample collection to the final PSP "score" calculation.

Sample Collection

Sample Stations

Since the appearance, progression, and duration of PSP toxin varies according to environmental and oceanographic features, the sampling stations for the state of Maine are divided into two major geographic regions. "Western Maine" stations, which include the area from the Maine/New Hampshire border to the middle of Penobscot Bay, are handled by the West Boothbay Harbor laboratory. "Eastern Maine" stations, which include the middle of Penobscot Bay to the Maine/Canadian border, are handled by the Lamoine laboratory (Fig. 1). For the off-shore mahogany clam fishery (*Arctica islandica*), a commercial vessel is contracted each year to take a DMR staff person out to pre-designated sample locations in the mahogany clam beds on a weekly basis, from April through October. For a complete table of all of the inshore and offshore sampling stations, see Appendix II.

Primary stations have been established throughout the entire coast of Maine. These stations were chosen because they are located at key areas which have historically "caught" the first signs of red tide. These stations are sampled on a weekly basis just before, during, and just after red tide season. The blue mussel, *Mytilus edulis,* is gathered at all of these primary stations. The soft-shell clam, *Mya arenaria*, is also collected at several of the primary stations.

During the onset, peak, and waning of red tide season, secondary and sometimes tertiary sampling stations are added, as well as the sampling of additional species, including surf/hen clams (*Spisula solidissima*), razor clams (*Ensis directus*), bay quahogs (*Mercenaria mercenaria*), American oysters (*Crassostrea virginicus*), and European oysters (*Ostrea edulis*). This expanded sampling program allows the Biotoxin Monitoring Program to get a much finer scale of resolution for toxicity levels in different areas and in different species, and allows for the surgical closures throughout the coast which will shut down shellfish beds that are contaminated with red tide toxin, while allowing clean shellfish beds to remain open for safe harvest.

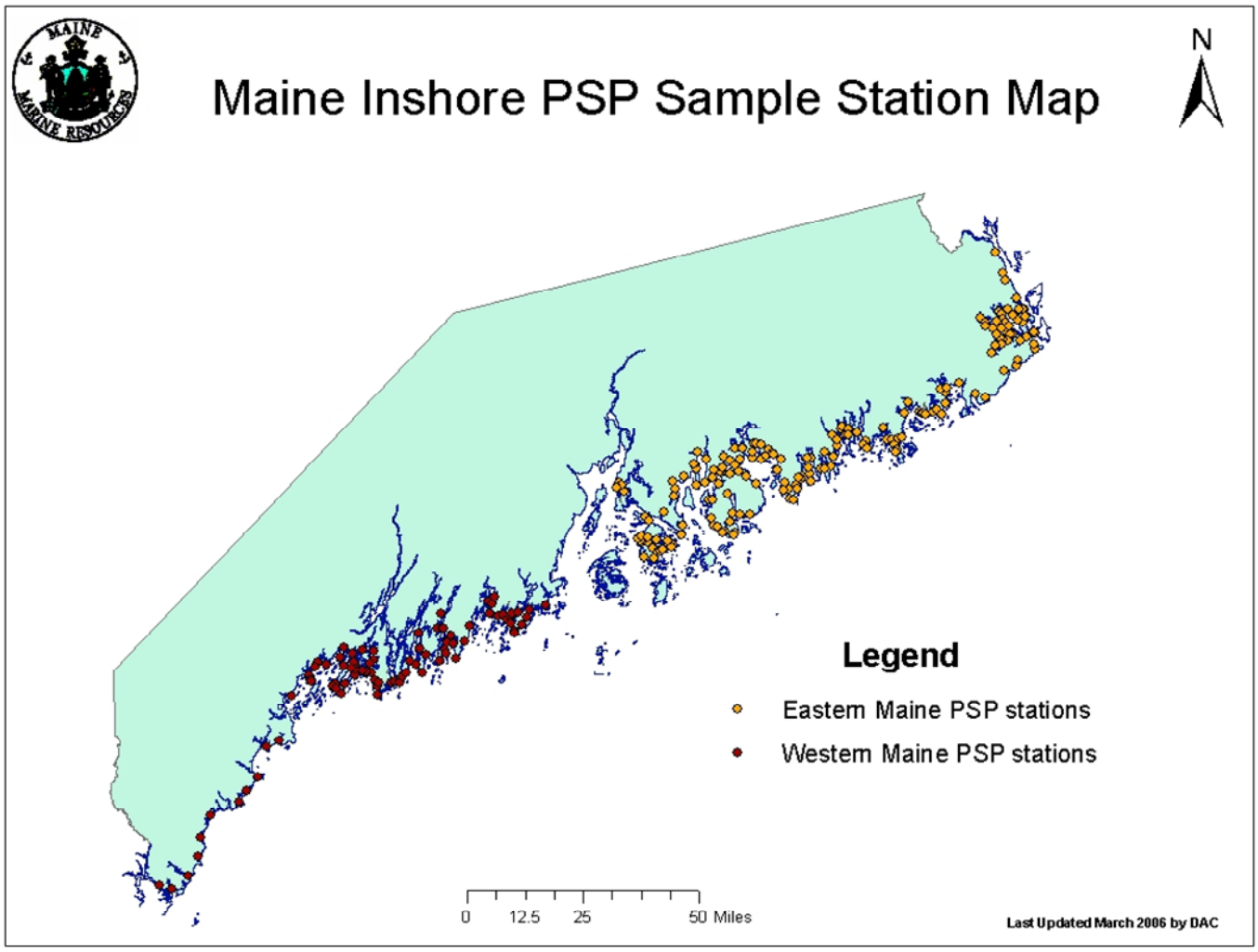


Figure 1. Maine PSP sampling stations.

Sample Collection

All collection of samples shall occur during the low tide, and samples shall be taken sub-tidally, or as close to the water line as possible. Due to the expected variation of toxin in individual shellfish, it is important to have a representative sample of each lot of shellfish. Shellfish samples shall contain at least 12 animals, and should yield 100 – 150 grams of shellfish tissue. Exceptions will be made in emergency situations when a sample is absolutely necessary and there is not enough stock at the sample site or when a sample of >12 animals fails to yield 100 grams of tissue. Samples shall be placed in a clean waterproof plastic bag. Sample bags must be identified with sample location, and the date of collection. Samples shall immediately be placed into an insulated ice chest (cooler) and packed on ice or with cold packs. A Field Data Sheet (Appendix I) must be filled out completely with the sampler's name, date, area number, sample location, genus, and time of collection, and shall accompany the samples until they are received by and logged into the laboratory. The samples must be delivered to the lab within 24 hours of collection.

Transportation

Upon collection, all samples shall be stored in insulated ice chests with ice or cold packs to maintain the temperature between 0° and 10° C until delivery to the lab. A thermometer must be kept in the cooler at all times, in order to monitor the temperature.

For chain of custody purposes, if samples are transferred to another individual, the Field Data Sheet which accompanies the samples must be signed by the person relinquishing custody and the person receiving custody, noting the date and time of transfer as well as the temperature. This process is also followed when the samples are received by the laboratory.

Laboratory Receipt

Upon arrival at the laboratory, the Field Data Sheet is filled in by the person relinquishing the samples and by the person receiving the samples, and the temperature is recorded at that time. Samples shall be placed in the sample refrigerator immediately upon arrival at the lab.

There shall be a sample login sheet that is located near the sample refrigerator. The log must be filled out completely with the date and time of receipt, temperature of the received samples, name of sample area, date of collection, number of samples, and collector's name.

Sample Processing

Sample Preparation

Prepare a bench card for each shellfish sample. In the center of the card, write the sample site, the date of sample collection, the species, and the area designation. In the upper left corner of the card, write the laboratory sample number; the laboratory number shall be a combination of

the day of the month, followed by a dividing line, then a consecutive series number. For example, three shellfish samples processed on July 23rd shall be assigned laboratory numbers as follows: 23/1, 23/2, 23/3.

Arrange one 500 ml beaker for each sample on the lab bench, and use a red permanent marker to label each beaker with the corresponding laboratory sample number. Spray the interior of each beaker with a light coating of dilute antifoam silicone emulsion. Place the corresponding sample card in each beaker.

Remove shellfish samples from the refrigerator, and place them in the correct order next to the shucking area.

Tissue Removal

1) Place one shellfish sample at a time in the rinsing screen. Separate shellfish, and rinse thoroughly with tap water.
2) Open each shell with a shucking knife, and cut the adductor mussel and any connective tissue as close to the shell as possible, in order to remove the whole animal from the shell without slicing into the main body tissue.
3) Once all the shellfish from the sample have been shucked, rinse the meats thoroughly with tap water to remove sand, shell, and foreign material.
4) Transfer the rinsed meats to a #10 mesh sieve, arrange in a single layer, and drain for 5 minutes.
5) Place the drained, shucked meats in a clean blender jar, and blend for 60 – 120 seconds to produce a uniform homogenate.

PSP Extraction

1) Select a prepared 500 ml beaker and remove the corresponding bench card. Weigh the beaker on a balance that is accurate to 0.1g, and record the weight of the beaker on the bench card.
2) Tare the balance with the beaker still in place, add 100g of the corresponding shellfish sample homogenate, and record the weight of homogenate on the bench card.

Note: If the sample size did not produce 100g of homogenate tissue, then place all of the available homogenate in the beaker and record the weight

3) Add 100g of 0.1 N HCL, and record the weight of added acid on the bench card.

Note: If the sample size from step 2 was less than 100g, then match the weight of the acid with the sample weight, to produce a 1:1 ratio.

4) Sum the total weight of the beaker, homogenate, and acid and record on the bench card.
5) Use a clean Teflon stirring rod to mix the homogenate and acid well. Check the pH with a strip of 0 – 2.5 and/or 2.5 – 4.5 paper, as necessary, to be sure that the pH is within the accepted range of 2 – 4. If the pH is less than 2, then stir the sample and adjust with one drop at a time of 0.1 N NaOH to bring up to the correct range. If the pH is greater than 4,

then stir the sample and adjust with one drop at a time of 5 N HCl to bring down to the correct range.

6) Place a 125mm Pyrex watchglass on top of the beaker, and place the beaker on a hotplate which has been pre-heated to 300^{o}F.
7) Monitor the sample carefully, stirring occasionally; as the mixture begins to warm up, insert a digital thermometer to track temperature. As the mixture approached boiling, then check the pH with a strip of 0 – 2.5 and/or 2.5 – 4.5 paper, as necessary, to be sure that the pH is within the accepted range of 2 – 4; if the pH is less than 2, remove from heat, and while stirring adjust with one drop at a time of 0.1 N NaOH to bring up to the correct range. If the pH is greater than 4, then remove from heat, and while stirring adjust with one drop at a time of 5 N HCl to bring down to the correct range. Once pH has been checked, remove the stirring rod.
8) When digital thermometer registers 100.0^{o} C ($\pm$ 1^{o} C), begin timing the extraction for 5 minutes. Wipe digital thermometer clean before placing in the next sample.
9) After 5 minutes of gentle boiling, use silicone mitts to remove beaker from hotplate and place on lab bench to cool. If rapid cooling is required, beaker may be placed in a shallow ice bath. Keep watchglass on beaker during the entire cooling process.
10) When sample has cooled to ambient room temperature, carefully remove the watchglass, allowing any condensation to drip back into the sample.
11) Stir the sample with a clean wooden applicator stick, and check the pH again with a strip of 0 – 2.5 and/or 2.5 – 4.5 paper, as necessary, to be sure that the pH is within the accepted range of 2 – 4; adjust if necessary, according to the method in step 5 of this section.
12) Weigh the sample on the balance. Check the total weight recorded on the corresponding bench card, and adjust for any evaporation loss by adding the necessary amount of 0.003 N HCL (pH 3).
13) Stir sample well, and pour an aliquot into a clean, dry centrifuge tube. Centrifuge at 3000 rpm (setting "5") for 5 minutes.
14) Remove tube from centrifuge unit, and place in the corresponding sample beaker.

Note: If samples will not be tested immediately, then decant the supernatant into a clean centrifuge tube, place inside the sample beaker, cover and refrigerate.

Bioassay

Extract Preparation

Extract should be at room temperature. Fill three 1ml 3/8" 26G disposable syringes with exactly 1ml of extract, being sure to eliminate any air bubbles.

Bioassay Set-up

Set up three 2000ml beakers per sample, and label each beaker with permanent marker in a consecutive series. Fill out a laboratory bench sheet with the beaker number, laboratory sample

number, sample site, the date of sample collection, the species, and the area designation. Place the prepared syringes next to the large beakers. Set a running timer to "0."

Injection

Select three mice for each sample. Each mouse should have been acclimated for at least 24 hours, and should weigh 17 – 23 g.

1) Pick up mouse by the tail, and weigh in a 200ml beaker (that has been tared) on a balance accurate to 0.1g. Record mouse weight (to the nearest 0.1g) on the bench sheet.
2) Hold mouse gently but firmly, keeping the legs and tail out of the way, and inject the mouse intraperitoneally in the lower right quadrant of the abdomen with 1ml of sample. Begin the timer, or note the exact injection time (to the nearest second) on bench sheet.
3) Check mouse for a "clean" injection – there should not be any sign of blood, and there should not be any bubbles between the skin and the peritoneum; if either of these conditions are present, the mouse should be discarded, a fresh needle should be prepared, and the injection should be redone with a fresh mouse.
4) Place the injected mouse in the corresponding beaker for observation.
5) If signs of PSP appear, note them on the bench sheet. If the mouse dies, record the exact time (to the nearest second) of the last gasping breath on the bench sheet.
6) If the median mouse died in less than 300 seconds, prepare an appropriate dilution sample (see **Dilutions** section below). If any mouse died after 300 seconds, place in the freezer compartment to await cremation at a later date. If any mouse did not die after 20 minutes, then the mouse shall be placed in a sealed 5 gallon plastic bucket and briefly gassed with CO_2 in order to euthanize the mouse. The mouse shall then be placed in the freezer compartment to await cremation at a later date.

Dilutions

For any sample in which the median mouse died in less than 300 seconds, an appropriate dilution must be prepared, and the sample shall be run again, until the median mouse dies within the appropriate time range.

Consult the death times in the Dilution Table (Appendix I) to determine the correct dilution concentration. If a death time falls within the range of two suggested concentrations, the least dilute option should be selected.

Mix dilutions gravimetrically (by weight), NOT by volume. For example, a 1:3 dilution should be prepared by taring a small plastic specimen cup, then using a clean, disposable pipet to measure 1.0g of extract, and another clean, disposable pipet to measure 3.0g of 0.003 N HCL. Mix the dilution well, and repeat the injection process, using mice in the 19 – 21g weight range. *Note: if there are no mice available in the 19 – 21g weight range, then the sample should be covered and refrigerated until the correct range of mice are available.* If the dilution was not "dilute" enough to achieve the minimum median death time of 300 seconds, then a fresh dilution should be prepared with the next ratio on the Dilution Table list. If a dilution was "too dilute,"

and the median mouse died after 420 seconds, then a fresh dilution should be prepared with the previous ratio on the Dilution Table list.

Calculations

At the end of a bioassay, consult the Sommer's Table and Weight Conversion Table to convert the calculated death time and the mouse weight to Mouse Units (MU), and record both of these numbers on the bench sheet. For any mouse that survived the bioassay test, the death time MU should be recorded as <0.875. On the same line, record the laboratory Conversion Factor (CF), and the total mass of the sample extracted. Finally, the Dilution Factor (DF) shall be calculated and written on the bench sheet; for any sample that was not diluted, the DF = 1, and is not required to be written on the bench sheet. If a dilution was mixed, the DF is calculated by adding 1 to the dilution amount; e.g., for a 1:3 dilution, the DF = 1 + 3 = 4.

The toxicity calculation for each replicate should be as follows:

Death time (MU) x Weight (MU) x CF x 200 x DF

The final toxicity "score" for the sample should be as follows:

Median Corrected Mouse Unit (MCMU) x CF x 200 x DF

Clean Up

At the end of the bioassay, dead mice shall be placed in the freezer storage compartment to await cremation at a later date, and live mice shall be placed in a sealed 5 gallon plastic bucket and briefly gassed with CO_2 in order to euthanize the mice. The mice shall then be placed in the freezer compartment to await cremation at a later date.

The solids shall be strained into a screen and discarded in the trash. Liquid extracts shall be rinsed down the lab sink. Beakers shall be pre-washed to remove any loose material, and may need to be scrubbed to remove tissue that remains. All glassware and all materials used during sample processing shall be placed in the dishwasher with a sanitizing detergent.

All surfaces in the lab shall be wiped down with a disinfecting cleaner.

Administrative Actions

Closures

Maine DMR laws and regulations allow for the immediate closure of areas that contain toxins known to be a public health threat, revoking of all shellfish licenses and permits in the event of a shellfish emergency, and allow the for the embargo, confiscation and destruction of contaminated shellfish.

When mouse bioassay (MBA) scores indicate that shellfish are approaching the federally mandated action quarantine level of 80µg/100g, the Director of the Biotoxin Monitoring Program will prepare a species-specific emergency legal notice to close that area to the harvest of shellfish. The size of the closure is determined based on the surrounding MBA scores, as well as the species tested, and any special circumstances, such as an approaching weather system or other oceanographic feature that may affect toxin distribution. A closure always includes a "safety zone," using stations that have been verified to be safely below quarantine level as the boundary location for the closure. If the status of a harvest area is suspected to be unsafe, it shall be closed until scientific data have been collected and have demonstrated that shellfish in the area are not a threat to public health.

The Director of the Public Health Division is immediately notified of the proposed legal notice, and Marine Patrol is consulted on any potential enforcement issues. The proposed legal notice and an accompanying map are presented to the Commissioner of the Maine Department of Marine Resources or his agents, and once it has been signed, it is immediately in effect. Copies of the legal notice and map are then distributed via fax, e-mail, and mail to all affected town offices, shellfish wardens, Marine Patrol officers, and an extensive list of shellfish industry members. The information about the closure is also added to the Shellfish Sanitation Hotline, which is a toll-free number that anyone may call to get updated information about the status of the shellfish harvesting areas. Legal notices are immediately added to the Department of Marine Resources website, and are printed in local newspapers.

Openings

When MBA tests in an area indicate that PSP is no longer a threat to public health, the Director of the Biotoxin Monitoring Program will prepare a species-specific emergency legal notice to open that area to the harvest of shellfish, and follow all of the procedures outlined in the **Closures** section. All MBA scores in the area to be opened must be below quarantine level for at least two consecutive tests, at least one week apart. If the status of a harvest area is unknown, and cannot be verified by regular MBA testing, it shall remain closed to protect public health.

Stock Solutions

0.04% Bromothymol Blue

1) In a 250 ml volumetric flask, weigh out 1g BTB.
2) Use a disposable glass pipet to add 16ml of 0.01N NaOH
3) Dilute to 250ml with deionized water.

5N HCl

1) Working under the fume hood, add 50 ml of deionized water to a 100ml volumetric flask.
2) Use a disposable glass pipette to transfer 42ml of 12N HCL to the flask.
3) Fill the remaining space in the flask to 100ml with deionized water. Cover securely with a glass stopper, and store at room temperature inside an acid cabinet.

0.1N HCl

1) Fill a 50 L Nalgene carboy with 50 L of deionized water.
2) Add 420ml of 12N HCl
3) Fill the remaining space in the carboy with deionized water. Cover securely and store at room temperature.

0.003N HCl ("pH 3 water")

1) Fill a 2L Nalgene flask halfway with deionized water.
2) Use a 100ml graduated cylinder to add 60ml of 0.1N HCl
3) Fill the flask to the 2L mark with deionized water.
4) Check the pH with a pH strip. The pH should equal "3." Cover securely and store at room temperature.

Silicon Spray

1) Add ~1 Tablespoon of Silicon Concentrate to a 1 L spray bottle.
2) Dilute with deionized water.

IV.

Internal Quality Control

Equipment Care and Maintenance

Balance Use and Calibration

Always use the balance in a clean, draft-free space, and check that the balance is clean and level. Adjust as needed by the front leveling feet. Balances should be cleaned after use or whenever a spill occurs

An annual calibration should be performed by the company currently under state contract to provide such services. Monthly checks of balance calibration should be done as follows:

1) In a clean, draft-free space, check that the balance is clean and level. Adjust as needed by the front leveling feet.
2) Retrieve Class I ASTM weights or NIST Ultra Class weights from the Water Quality department.
3) Turn the balance on, and allow it to stabilize.
4) Using the provided forceps to handle the weights, place the 50g weight on the scale, allow it to stabilize, and record the weight in the Quality Assurance Tests binder.
5) Using the forceps, return the weight to the box.
6) Repeat the previous step with the 100g, 0.1g, and 2000g weights, respectively.

The balance must provide a sensitivity of 0.1 gram at a load of 150g; if the balance is discovered to be off by more than 0.1g, then it shall be placed out of service, and a properly functioning balance shall be borrowed from another department until the problem can be corrected. If a repair cannot be made, the unit shall be replaced.

Blender

The blender should be cleaned after every use. The blender jar should be washed in the dishwasher and the base cleaned with a cleaning solvent. The blades should be examined regularly to insure proper use and safety. If the blender appears to be working improperly, a qualified technician is called in to perform any necessary repairs. If a repair cannot be made, the unit shall be replaced.

Centrifuge

The centrifuge should only be operated with a balanced load of samples. The centrifuge should be taken apart and cleaned monthly or whenever there is a spill. If the centrifuge appears to be working improperly, a qualified technician is called in to perform any necessary repairs. If a repair cannot be made, the unit shall be replaced.

Deionization System

The deionizer must be checked monthly bacteria, and annually for metals. These checks are performed by the Water Quality staff. Results are recorded in the Quality Assurance Tests binder.

Once a month the resistivity is checked and documented. The resistivity should be between 10 and 18, exceeding 0.5 megohms resistance. Results are recorded in the Quality Assurance Tests binder.

Once a month a chlorine tablet is added to the system, and the system is and flushed three (3) times.

If the deionozer system fails any of these standards, a qualified technician is called in to perform any necessary repairs. If a repair cannot be made, the unit shall be replaced.

Detergent Residue Test

Once per day of activity in the lab, a detergent residue test must be performed.

1) Randomly select several pieces of glassware at the completion of a wash cycle.
2) When glassware is completely dry, place a few drops of 0.04% Bromothymol Blue on or in each piece, and observe to see if there is a color change; Bromothymol Blue is greenish blue when applied, and will turn yellow if an acid is present, or blue if a base is present. There will be no color change if the glassware is neutral.
3) Record any color change in the Quality Assurance Tests binder.

Dishwasher

Dishwasher should be operated with approved sanitizing detergent. Sprinkle approximately 1 tablespoon of detergent on the inside of the door of the unit, then close the door and allow the cycle to run. If there appears to be any detergent residue on the glassware after one complete cycle, then run the dishwasher for an additional cycle, without adding any detergent. If the dishwasher is not functioning properly, a qualified technician is called in to perform any necessary repairs. If a repair cannot be made, the unit shall be replaced.

Fume Hood

The fume hood has a draw of 100 at 15 inches with a standard accuracy of 20. A qualified technician shall check the flow on the fume hood annually. Calibration records are maintained in the Quality Assurance Tests binder. If the hood flow rate drops below the standard accuracy, then a qualified technician shall be brought in to resolve the situation. If a repair cannot be made, the unit shall be replaced.

Glassware

Only borosilicate glassware should be used, and whenever possible Class A glassware should be used. All chipped and broken glassware shall be disposed of properly. All glassware must be

rinsed with tap water after use and before dishwashing. Glassware must be randomly checked for soap residue after the dishwasher has been run.

Hotplate

Hotplate should be turned on to preheat for at least 30 minutes before any sample processing occurs. Hotplate should be turned at the end of each day of processing. If the hotplate is not heating up correctly or maintaining temperature, a qualified technician is called in to perform any necessary repairs. If a repair cannot be made, the unit shall be replaced.

Pipettes

Only disposable, borosilicate glass or non-toxic polystyrene pipettes should be used. Pipettes should not exceed an error of 2.5 %. Pipettes should deliver readily and accurately and are permanently graduated with unbroken tips. Pipettes larger than 10 ml are not to be used to deliver 1.0 ml aliquots. Pipettes larger than 1 ml are not to be used to deliver 0.1 ml aliquots. A silicone bulb is always used with the pipettes (NEVER use your mouth to pipette).

Refrigerator

Refrigerator temperature is maintained between 0 and 4 °C. Refrigerator temperature is checked at least once daily. Temperature records are maintained on a monthly record sheet located on the refrigerator, and upon completion, the monthly the record is placed in the Quality Assurance Tests binder. If the temperature is not within the acceptable limits, a manual adjustment may be made of the temperature control. If this adjustment does not correct the problem, a qualified technician shall be contacted to repair the unit. If a repair is not successful, the unit shall be replaced.

.**Safety Equipment**

Eyewash stations and safety showers are checked each month by operating the valves to check for proper function. Fire extinguishers are examined to ensure the gauge indicates that it is operational. Records are maintained in the Quality Assurance Tests binder. If the safety equipment is not working properly, a qualified technician shall be contacted to repair the unit. If a repair is not successful, the unit shall be replaced.

Syringes

Only disposable, 1ml, 3/8" 26G syringes shall be used for any work in the bioassay. After use, syringe tips shall be snapped off into a closed, heavy-duty plastic container, and the syringe body shall be disposed in the laboratory garbage can.

Thermometers

For general purposes appropriately ranged thermometers shall be used. All equipment being monitored for temperature should have a chart located on or near it on which temperature should be logged daily. The accuracy of all thermometers should be determined by comparison with a certified National Bureau of Standards (NBS) thermometer.

Weekly Conversion Factor Check

Each week that a bioassay is run, the lab staff shall perform a weekly Conversion Factor (CF) Check. The CF check shall be performed as follows:

Prepare the Working Standard Solution

1) Open a new STX ampule, recording the FDA reference number and the date on the Working Standard Solution worksheet.
2) Tare a clean, dry 100ml volumetric flask. Use a disposable 1ml pipette to transfer 1ml of STX to the flask.
3) Fill the flask to 100ml with pH3 water.
4) Place a suitable cap on the flask, shake well, and label the flask with the FDA reference number and the date.
5) Once the Working Standard Solution has been prepared, it shall be stored in the refrigerator, and may be used as long as it can produce a median death time of 5 – 7 minutes (typically up to six months).

Prepare the CF Dilution

1) Remove the Working Standard Solution from the refrigerator. Wipe away any exterior condensation.
2) Weigh the flask, with cap in place, and record the date and weight on the Working Standard Solution worksheet.
3) Tare a clean, dry plastic specimen cup. Use a disposable 10ml glass pipette to measure out 10g of Working Standard Solution.
4) Place cap on Working Standard Solution, and weigh again. Record new weight in the Working Standard Solution worksheet. Return Working Standard Solution to the refrigerator.
5) Check with the Scientist to determine the correct dilution; the lab may use a 10:19, a 10:20, or a 10:21 dilution.
6) Use a disposable 10ml glass pipette to measure out the correct amount of 0.003N HCl ("pH 3 water"). For a 10:19 dilution, add 19g pH3 water. For a 10:20 dilution, add 20g pH3 water. For a 10:21 dilution, add 21g pH3 water. Record dilution used on the CF worksheet.
7) Stir the CF Dilution well with a Teflon rod, and check the pH with a pH strip. Record the pH on the CF worksheet.

CF Check Bioassay

1) Stir the CF Dilution well, and fill five disposable 1ml 3/8" 26G syringes with exactly 1ml of solution, being sure to eliminate any air bubbles.
2) Select five mice for the CF Bioassay; mice must be between 17 – 23g, and it is preferable to use mice between 19 – 21g.

3) Pick up mouse by the tail, and weigh in a 200ml beaker (that has been tared) on a balance accurate to 0.1g. Record mouse weight (to the nearest 0.1g) on the CF worksheet.
4) Hold mouse gently but firmly, keeping the legs and tail out of the way, and inject the mouse intraperitoneally in the lower right quadrant of the abdomen with 1ml of sample. Begin the timer, or note the exact injection time (to the nearest second) on CF worksheet.
5) Check mouse for a "clean" injection – there should be no sign of blood, and there should not be any bubbles between the skin and the peritoneum; if either of these conditions is present, the mouse should be discarded, a fresh needle should be prepared, and the injection should be redone with a fresh mouse.
6) Place the injected mouse in the corresponding 2000ml beaker for observation.
7) Record the exact time of death (to the nearest second) of the last gasping breath on the CF worksheet.
8) Clean up shall be done in accordance with the methods outlined in the Standard Operating Procedures – Bioassay section.

CF Check Calculation

1) At the end of the bioassay, consult the Sommer's Table and Weight Conversion Table to convert the calculated death time and the mouse weight to Mouse Units (MU), and record both of these numbers on the CF worksheet. Calculate the Corrected Mouse Units as follows:

Death time (MU) x Weight (MU) = Corrected Mouse Units (CMU)

2) Record the CMU on the CF worksheet.
3) Number the CMU in order of increasing value, and circle the median CMU. Record the median CMU on the CF worksheet in the space labeled "MCMU."
4) Consult the Sommer's Table, and find the death time that corresponds to the MCMU. Record this time on the CF worksheet in the space labeled "Median death time."
5) Calculate the Conversion Factor as follows:

$$\mathbf{CF = \frac{(Dilution\ of\ reference\ solution)}{(MCMU)}}$$

6) Check the calculated CF against the acceptable range for the laboratory CF. The CF should be $\pm$ 20% of the current laboratory CF. As of August, 2006, the laboratory CF = .21. Note if the laboratory CF was verified or not on the CF worksheet.
7) If the CF check was not verified, then five additional mice should be injected, to make a group of ten mice. Another group of ten additional mice should also be injected. The CF for each group of ten must be calculated, and the average of the two CF calculations should be used for the bioassay calculations of that week. All subsequent bioassays should continue to use the verified CF value; if this value fails to be verified more than three times in a year, and the cause cannot be determined, then a new laboratory CF standardization must be done.

Data and Documentation

Document Control

Records are maintained in a manner such that any employee can duplicate the contents. All records are maintained in properly identified folders or binders. All records are written in ink, and are legible and accurate. Erroneous entries and all changes will be crossed out by a single line and initialed by the analyst. All records must be dated. All QA/QC documentation including evaluations, audits, and monitoring logs will be maintained in the QA/QC binder. The responsibility for accurate documentation of procedures and data rests with each analyst.

Data Reduction

Properly trained staff members are responsible for converting data into final units using appropriate procedures. Supervisors are responsible for checking at least 10% of staff calculations.

Validation

Supervisors are required to check 10% of all QC criteria. When a new employee joins the staff, that employee shall be subject to 100% check on all QC criteria for the first 30 days, and the percent of checks shall be gradually reduced to 10% over a six-month period.

Reporting

Staff will report results on bench cards, Mouse Bioassay Recording Sheets, and on Biotoxin Monitoring Field Sheets.

Verification

Scientists are responsible for ensuring accuracy of reports generated by volunteers, staff and other state officials.

Test results go through several transcriptions, which must be verified. Sample information on reports must be reviewed for completeness and accuracy. Computer data sheets must be checked for completeness and accuracy.

The current calendar year's quality control records are maintained in binders in the PSP office/lab. Records from prior years are bound together and stored in the file boxes in the PSP Office.

V.

Laboratory Safety

General Safety Precautions

All personnel should be aware of general safety measures in the laboratory including: evacuation routes, eyewash, and safety shower locations, location and proper handling of chemicals, and proper handling of biotoxins.

Saxitoxin is known to be a potent sodium channel blocker. Use proper safety measures and read the appropriate MSDS for handling requirements.

Up-to-date immunization for tetanus prevention is recommended for each analyst, and all persons handling mice.

No eating or drinking in areas where biotoxins may be present.

Wear shoes at all times in the lab. Do not wear sandals or open-toed shoes.

Do not store food or beverages in refrigerator, freezer, glassware, or utensils used for lab operations.

All personnel are responsible for ensuring that the laboratory is cleaned after the extraction and assay is complete.

Reagents and equipment should be returned to their proper places after use.

Countertops should be kept neat and clean.

Use a safety pipetting device for pipetting samples, and reagents. No mouth pipetting.

Discard broken glassware properly.

Spills shall be cleaned immediately.

Keep work area clean, uncluttered, and disinfected.

Wear gloves when handling samples and potentially toxic chemicals.

Wear appropriate eye protection when working with chemicals.

Wear appropriate clothing (aprons) when working in the laboratory.

Lifting of heavy objects must be performed using the legs to lift and not the back.

Mats shall be kept in good condition.

All working surfaces and floors should be cleaned regularly.

All containers must be labeled with the identity of the contents.

All employees shall be instructed in:

Safe handling procedures involving gas cylinders and regulators

Awareness of tidal stages and attention to shoreline conditions

Proper techniques for use and disposal of needles used in the bioassay

Boothbay Harbor – working in teams when surf-clamming

Safety Equipment

Fire Extinguishers

Fire extinguishers must be clearly labeled to indicate the types of fire they are designed to extinguish.
Class A – ordinary combustible materials such as wood, cloth, paper, rubber, and plastics.
Class B – flammable liquids such as oils, greases, tars, lacquers, and also flammable gases.
Class C – energized electrical equipment.
Class D – combustible metals such as magnesium, titanium, sodium, and lithium.

Halon type extinguishers are designed to leave no residue. These types should be used around instruments or computers. After the use of a halon type extinguisher, be sure to adequately ventilate the room before reentering.

Fire extinguishers should never be concealed or blocked from easy access.

Safety Showers

If all protective measures fail and an employee is involved in a chemical spill, a safety shower is located in the lab for immediate use.

Employees should be familiar with the location of the safety shower and how to operate it properly. An employee should stand under the shower and activate the shower. They should remain there flooding the affected area for 15 – 30 minutes. If the chemical spill is corrosive the employee should remove his/her clothing.

The appropriate supervisor should be notified when an employee is required to use the safety shower.

Injuries should be documented as “work related” should the injury lead to later more serious complications.

Eyewash Station

If all protective measures fail and an employee gets a foreign object or a chemical in the eye, an eye wash station is located in the lab for immediate use.

Employees should familiarize themselves with the location and operation of the eyewash. Always flood the eyes for at least 15 minutes to insure complete removal of the object or chemical. Flush from the inner eye outward.

The appropriate supervisor should be notified when an employee is required to use the eyewash.

Injuries should be documented as “work related” should the injury lead to later more serious complications.

First Aid Kit

The first aid kit should be located in an obvious place in the laboratory, and is to be used for minor injuries.

First aid kits should contain band-aids, sterile gauze pads, antiseptic wipes, and burn ointment.

Minor injuries requiring first aid should be reported to the appropriate supervisor.

Injuries should be documented as “work related” should the injury lead to later more serious complications.

Fume Hood

Work that involves noxious materials which are toxic, odiferous, volatile, or harmful shall be conducted within the fume hood.

The purpose of the fume hood is to keep toxic or irritating vapors and fumes out of the general laboratory working area.

Always make sure the exhaust fan is operating when working under the hood.

**While working under the hood the sliding sash should be kept at a height designed to ensure a safe draw of the air out of the hood.

**When not in use the sash of the hood should be kept closed.
**N/A in Boothbay lab for assaying samples

Flammable-Liquid Storage Cabinet

This cabinet is designed for the storage of flammable liquids and should be used and maintained by the manufacturer's specifications.

Store only compatible materials inside.

Do not store paper or other combustibles inside the cabinet.

Do not overload the cabinet.

Acid Storage Cabinet

This cabinet is designed for the storage of acidic liquids and should be used and maintained by the manufacturers specifications.

Store only acids inside.

Do not overload the cabinet.

Eye Protection

Eye protection is mandatory in all areas where there is a potential for injury. For most situations safety glasses with side shields are adequate. Where there is a potential danger of splashing chemicals goggles are required.

Clothing

Since chemicals are used regularly and the shellfish extraction process can be wet and messy, plastic aprons should be worn for protection and convenience. Each employee is responsible for cleaning and maintaining their own apron.

Gloves

When handling chemicals or potentially toxic shellfish gloves should be worn. When shucking shellfish latex/nitrile surgical gloves should be worn to protect against possible PSP exposure. When handling chemicals neoprene or nitrile gloves should be worn. Both types of gloves are provided by the department.

Injuries and Other Emergencies

Personal Injuries

Personal injury is not uncommon in the laboratory or in the field. Some injuries are minor and only require first aid. Other injuries may need medical attention. EVERY injury must be reported to a supervisor, immediately assessed and, if needed, medical help should be

summoned. Emergency numbers should be posted on or beside the telephone in the laboratory. Emergency numbers should be kept in each sampling vehicle along with a cellular phone. After calling for medical help the appropriate supervisor should be contacted and Hallowell notified.

Employees should understand that the purpose of reporting and documenting accidents and injuries is not to place blame but to determine cause so that similar accidents may be prevented. The program supervisor and the laboratory director should investigate all accidents. Tom Cotnoir will be copied on all injury reports.

Fires

In the event of a fire assist any person in immediate danger, and immediately activate the fire alarms. If the fire is small use a nearby fire extinguisher to put out the fire. If the first attempts to put out the fire are unsuccessful leave the fire and evacuate the building. The local fire department should be notified to handle the blaze. The lab director is responsible for accounting for all personnel. All fires should be reported to the program director. Every employee should be familiar with the building Fire Evacuation Plan.

Compressed Gas Cylinders

Occasionally, a cylinder or one of its component parts develops a leak at the top of the cylinder in the valve threads, stem, or outlet. If a leak is suspected soapy water or other "snoop" solutions should be used to detect the leak. Once the leak has been detected the packing nut should be tightened to try to stop the leak. If this does not stop the leak, than close the safety valve and consult the supplier for further instructions.

Material Safety Data Sheets

Material Safety Data Sheets (MSDS)

MSDS's for chemicals used and stored in the laboratory can be found in a labeled three ring binder in the laboratory. Before working with any unfamiliar chemical, all employees should consult the MSDS for that chemical. It is the responsibility of all employees to familiarize themselves with the contents of the MSDS binder.

VI.

Quality Assessment

Quality Assessment

All data entry and calculations are proofed by the Scientist I in Boothbay Harbor at year-end.

Laboratory staff are evaluated yearly using the State of Maine's Performance Appraisal system. Copies are discussed with and given to employees and maintained on file in the Department's Hallowell office.

Laboratory compliance with the National Shellfish Sanitation Program is currently overseen by the Food and Drug Administration, which conducts yearly evaluations. .

VII.

Proper Animal Care

Only Female CD-1 strain mice shall be used. All mice are ordered from the Charles River Company. Routinely mice are ordered from the Charles River Laboratories on Friday for the following Wednesday delivery. In emergency situations mice can be ordered for overnight delivery.

Mice are delivered to the Boothbay Harbor Laboratory in an air-conditioned van to maintain a comfortable temperature. Mice which will be used by the Lamoine laboratory are packed into an air-conditioned car and delivered to the Lamoine laboratory by a staff member.

After arrival at their final destination, the mice are removed from the shipping containers and placed in the small animal cages, ~20 mice per cage. The cages are layered with shavings and the mice are given ample food and water. The cages are then placed in the designated mouse house in the designated mouse room. The temperature is controlled to maintain a comfortable temperature.

Mice boxes are changed every other day with new shavings and fresh water. Food and water are checked daily.

Mice that are over the maximum injection weight are euthanized with CO_2 gas immediately. All injected mice that do not die during the bioassay are euthanized with CO_2 gas after the completion of the bioassay.

All dead mice are placed in the designated biohazard freezer. When the freezer is full, the mice are cremated by a contracted veterinary service.